Review

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Cell competition in intratumoral and tumor microenvironment interactions

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Abstract

Tumors are complex cellular and acellular environments within which cancer clones are under continuous selection pressures. Cancer cells are in a permanent mode of interaction and competition with each other as well as with the immediate microenvironment. In the course of these competitive interactions, cells share information regarding their general state of fitness, with less-fit cells being typically eliminated via apoptosis at the hands of those cells with greater cellular fitness. Competitive interactions involving exchange of cell fitness information have implications for tumor growth, metastasis, and therapy outcomes. Recent research has highlighted sophisticated pathways such as Flower, Hippo, Myc, and p53 signaling, which are employed by cancer cells and the surrounding microenvironment cells to achieve their evolutionary goals by means of cell competition mechanisms. In this review, we discuss these recent findings and explain their importance and role in evolution, growth, and treatment of cancer. We further consider potential physiological conditions, such as hypoxia and chemotherapy, that can function as selective pressures under which cell competition mechanisms may evolve differently or synergistically to confer oncogenic advantages to cancer.

Keywords cancer; cell competition; chemotherapy; clonal selection; tumor heterogeneity

Subject Categories Cancer; Cell Adhesion, Polarity & Cytoskeleton; Development

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Introduction

Cell competition (CC) is an active cell selection mechanism that promotes elimination of the viable but suboptimal cells in the presence of more competitive neighbors. The primary observations of CC were drawn from experiments investigating the impact of a class of mutations in ribosomal protein (Rp) encoding genes termed Minutes in the developing *Drosophila* wing disk (Morata & Ripoll, 1975). Cells with Rp heterozygous mutations were viable on own but had lower division capacity due to reduced protein synthesis compared to their wild-type (WT) counterparts. In genetic mosaic wing disks comprised of WT and MT cells, the wing disk is overtaken by the fast-dividing WT clones (winners), suggesting clones with higher division potential grow at the expense of their growth-deficient neighbors (losers) (Morata & Ripoll, 1975). Elimination of the weaker populations proceeds through apoptosis (Prober & Edgar, 2000; Moreno *et al*, 2002), specifically at the interface with their faster growing counterparts, suggesting CC is an active process enacted by short-range cellular interactions (Moreno *et al*, 2002). The same phenomena of CC-based elimination of growth-deficient cells were demonstrated in mice (Oliver *et al*, 2004), evincing CC as a highly conserved and important mechanism employed by multicellular organisms. CC is now understood to act as a result of fitness comparisons, in which cellular fitness status is qualified by various parameters not limited to growth. Importantly, competition hinges upon relative and not absolute fitness and is determined by cues of the tissue microenvironment (Moreno *et al*, 2002; Rhiner *et al*, 2010). This is particularly highlighted by the demonstration of supercompetition, in which normal WT cells are outcompeted by highly competent neighbors. This facet of CC was identified in the

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fly wing by overexpression of the *Drosophila* homolog of Myc (*dMyc*), a master regulator of cell growth and division, which resulted in apoptosis of WT cells and compensatory proliferation of fast-growing *dMyc*-overexpressing supercompetitors (Moreno & Basler, 2004). CC as a mode of quality control plays important roles in the selection for the most robust cell populations during development (Claveria *et al*, 2013; Diaz-Diaz *et al*, 2017; Hashimoto & Sasaki, 2019), organ size control (de la Cova *et al*, 2004), and maintenance of tissue homeostasis to regulate tissue regeneration (Oertel *et al*, 2006), and aging (Merino *et al*, 2015; Liu *et al*, 2019a). Studies on CC have offered important insights into how distinct cellular populations respond to each other and have provided a unique lens to explain various facets of cancer biology (Eichenlaub *et al*, 2016; Suijkerbuijk *et al*, 2016; Di Giacomo *et al*, 2017; Madan *et al*, 2019b; Moya *et al*, 2019). In this review, we discuss the basic mechanisms of homeostatic CC and how subversion of these mechanisms can result in cancer. We further focus on the recent discoveries that reveal the role of CC in a) CC between tumor and its microenvironment and b) intratumoral CC. In this review, we summarize the instances where CC is involved in the interactions between tumor and peritumoral cells, as they are crucial to the initial events of tissue transformation and subsequent tumor outgrowth and invasion. Additionally, we hypothesize that due to intratumoral CC, distinct cancer clones become more competent under selection pressures such as chemotherapy and this results in a more aggressive tumor with enhanced therapy resistance.

Cell competition general mechanisms

Since the original findings of CC identified in *Drosophila*, several additional studies have elaborated on key concepts such as how cells recognize fitness and trigger CC, genes and pathways involved in CC, and the various modes of elimination of the suboptimal cells (Table 1). The following mechanisms are discussed herein.

The trophic factor theory

This theory posits that homeostatic cell density is achieved by competition for the limited signaling cues in a tissue space. Herein, the more fit cells (winners) have a higher capacity to obtain growth/pro-survival signals and thus proliferate compared to their less-fit neighbors (losers), which undergo apoptosis. In the *Drosophila* wing, Rp-WT cells outcompete their slower-growing Rp-MT neighbors for decapentaplegic (Dpp) survival factor, a crucial morphogen for cellular division, tissue growth and correct patterning. Deficient Dpp signaling in Rp-MT cells resulted in lowered proliferation and activation of JNK-induced apoptosis (Moreno *et al*, 2002) (Fig 1A). Rp-MT cells display lowered protein translation rates (Moreno *et al*, 2002) and thus presumably reduced metabolic activity, which may account for diminished ability to capture Dpp ligands. In agreement, additional work has shown that loser cells displayed lower protein synthesis and a reliance on autophagy in association with downstream apoptosis via JNK signaling and Hid activation (Nagata *et al*, 2019). Mouse embryonic stem cells lacking the bone morphogenetic (BMP)-receptor are non-responsive to secreted BMPs, the mammalian homologs of Dpp, are deemed defective, and are outcompeted by WT neighbors. However, these losers did not display defects in autophagy (Sancho *et al*, 2013). Additionally, the dependency of extracellular growth cues to initiate competition is in question given the inconclusive role of competition for Dpp capture in *dMyc*-mediated CC (Moreno *et al*, 2002; de la Cova *et al*, 2004). And secondly, indirect competition based on trophic theory does not account for compensatory hyperproliferation of winner cells subsequent to apoptosis of loser cells.

One study showed just how homeostatic CC maintains tissue fitness status quo and suppresses dysfunction--and specifically how this is achieved based on affinity for environmental cues. The ability to transduce IL-7 signals was found to select youthful T-cell progenitors at the expense of old progenitors in the mouse thymus. Lack of the Il-7-receptor on young progenitors obviates this mode of CC and results in accumulation of the old T-cell progenitors, leading to the development of T-cell acute lymphoblastic leukemia (T-ALL) (Martins *et al*, 2014). This demonstrates that loser cells, by responding poorly to growth and survival factors present in the extracellular environment, are outcompeted by winner cells, independently of direct cell–cell interactions (Table 1).

Mechanical cell competition

Cells in any tissue space are members of a mechanical environment generated by forces from cell–cell contacts and the surrounding extracellular matrix. Slower or faster growing clones, relative to their surrounding neighbors, experience mechanical stress (Shraiman, 2005). Tissues have adopted various mechanisms to relieve this stress by balancing proliferation with elimination. Mechanical CC regulates the selection of the eliminated cells and, in some contexts, operates as a form of supercompetition (Levayer *et al*, 2016). In the *Drosophila* notum, cellular overcrowding, driven by conditional expression of activating RasV12 mutations, resulted in the compaction and subsequent outcompetition of neighboring WT cells via apoptosis, followed by delamination. Unlike other forms of CC described, this mode of mechanical supercompetition was not locally constrained and delamination was observed up to three cell diameters away from RasV12-mutant clones (Levayer *et al*, 2016). Later work showed that mechanical CC eliminated the normal cells in between RasV12-mutant clones via compaction-driven downregulation of epidermal growth factor (EGFR)/extracellular-regulated kinase (ERK) signaling. Tissue relaxation or densification also resulted in ERK downregulation, which suggests that ERK is not modulated simply by cellular density, but instead tissue strain rate (Moreno *et al*, 2019). In mammalian cells, polarity-deficient MDCK cells generated by knockdown of the apicobasal polarity and tumor suppressor gene, *Scribble* ($Scrib^{KD}$), were hypersensitive to compaction and were eliminated via apoptosis upon cellular overcrowding. Compaction of $Scrib^{KD}$ relayed a mechanical stress response via Rho kinase (ROCK) and actin and myosin upregulation, in line with previous studies showing cytoskeletal proteins interpret and respond to mechanical stress in apoptotic epithelia (Rosenblatt *et al*, 2001). Eliminated MT cells displayed ROCK-induced p38 stress signaling concomitant with high p53 expression, indicating that through compaction, mechanical CC supports removal of damaged cells (Wagstaff *et al*, 2016), potentially exploiting p53 as a sensor (Table 1). Altogether, these studies suggest that cells may have differential sensitivity to compaction that depends on their sensitivity to apoptosis. Mechanical CC seems to exploit these parameters to eliminate the relatively less-fit population. Thus far, mechanical CC does not appear to hinge upon

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Table 1. Summary of CC pathways and model systems.

Gene	CC type	Animal model	Tissue type/ organ	Described in cancer	Molecular mechanism	References
Dpp	Trophic factor theory	*Drosophila*	Wing disk	No	Rp-MT are outcompeted by Rp-WT owing to reduced Dpp-mediated anti-apoptotic signaling	Moreno *et al* (2002)
Il-7	Trophic factor theory	Mouse	Thymus	Yes (T-ALL)	Old (loser) progenitor cells are replaced by young (winner) progenitor cells by competing for IL-7	Martins *et al* (2014)
Scribble	Unknown mechanism	Canine	MDCK Cells	No	Scribble-deficient cells are engulfed by WT cells with JNK-activated phagocytic pathways	Ohsawa *et al* (2011)
Scribble	Mechanical CC	Canine	MDCK Cells	No	ROCK/p38/p53 mediated elimination of *Scribble* knockdown cells by surrounding WT cells	Norman *et al* (2012) Wagstaff *et al* (2016)
RasV12	Mechanical CC	*Drosophila*	Pupal notum midline	No	RasV12 cells induce compaction and elimination of WT cells through the downregulation of ERK signaling in loser cells	Moreno *et al* (2019)
RasV12	Mechanical CC	Canine	MDCK cells	No	RasV12-transformed cells undergo in Warburg-like metabolic changes when surrounded by WT cell, which promote their elimination through apical extrusion	Kon *et al* (2017)
RasV12	Mechanical CC	Mouse	Intestine epithelium	No	RasV12 cells harbor metabolic changes that promote their apical extrusion by winner cells	Kon *et al* (2017)
RasV12	Mechanical CC	Canine	MDCK cells	No	Filamin and vimentin mediated extrusion of RasV12- and Src-transformed loser cells by WT winner cells	Kajita *et al* (2014)
Src	Mechanical CC	Canine	MDCK cells	No	Filamin and vimentin mediated extrusion of RasV12- and Src-transformed loser cells by WT winner cells	Kajita *et al* (2014)
Src	Mechanical CC	Zebrafish	Embryos	No	Filamin and vimentin mediated extrusion of RasV12- and Src-transformed loser cells by WT winner cells	Kajita *et al* (2014)
Sas-PTP10D	Activation of receptor-mediated cell death	*Drosophila*	Imaginal disks	No	Transactivation of Sas-PTP10D signaling leads to *Scribble* MT cell elimination through JNK apoptotic signaling	Yamamoto *et al* (2017)
TRRs	Activation of receptor-mediated cell death	*Drosophila*	Wing disk, imaginal disks	No	The secreted factor *Spatzle* activates TRRs in $RpL14^{-/+}$ cells, leading to the activation of NF-kB-mediated apoptosis	Meyer *et al* (2014), Alpar *et al* (2018), Byun *et al* (2019), Katsukawa *et al* (2018)
dFwe	Fitness fingerprints	*Drosophila*	Imaginal disks	No	Fwe(Ubi) eliminates Fwe(Lose) cells via activation of Azot- and Hid-mediated apoptosis	Rhiner *et al* (2010), Merino *et al* (2015)
hFWE	Fitness fingerprints	Human	Cancer cells, xenografts	Yes (multiple)	Fwe-Win eliminates Fwe-Lose cells via activation of apoptosis	Madan *et al* (2019)
SPARC	Fitness Fingerprints	*Drosophila*	Imaginal disks	Yes	SPARC transiently inhibits the activation of caspase in Flower-Lose cells	Portela *et al* (2010)
dMYC	Unknown mechanism	*Drosophila*	Wing disk	No	MYC-high cells eliminate through apoptosis neighbor MYC-low cells	Moreno and Basler (2004)
c-MYC	Unknown mechanism	Mouse	Embryo (epiblast)	Yes	MYC-high cells eliminate through apoptosis neighbor MYC-low cells	Claveria *et al* (2013)
c-MYC	Unknown mechanism	Human	Cancer cells	Yes	MYC-high cells eliminate through apoptosis neighbor MYC-low cells	Di Giacomo *et al* (2017), Patel *et al* (2017)
Yki	Unknown mechanism	*Drosophila*	Wing disk	No	Yorkie (YAP homolog) expression activates dMyc promoting supercompetition causing the elimination of WT cells	Ziosi *et al* (2010)
YAP/TAZ	Unknown mechanism	Mouse	Glioblastoma	Yes	Glioblastoma cells with high expression of YAP outcompete cells with lower expression	Liu *et al* (2019)

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Table 1 (continued)

Gene	CC type	Animal model	Tissue type/ organ	Described in cancer	Molecular mechanism	References
YAP/TAZ	Unknown mechanism	Mouse	Liver	Yes	Peritumoral cells with high YAP/TAZ activation induce apoptosis in liver tumor cells with relatively less YAP/TAZ to suppress tumor outgrowth. High YAP/TAZ-liver tumor cells can also outcompete low-expressing normal hepatocytes and result in tumor growth.	Moya *et al* (2019)
YAP/ TEAD	Unknown mechanism	Mouse	Embryo (epiblast and embryonic fibroblasts)	No	Embryonic cells with high YAP/TEAD activity eliminate their neighbors with lower YAP/TEAD activity	Hashimoto and Sasaki (2019), Mamada *et al* (2015)
Wingless	Unknown mechanism	*Drosophila*	Imaginal disks	No	Cells with Wingless (WNT homolog) overactivation eliminate surrounding cells with low Wingless activity	Vincent *et al* (2011)
WNT	Unknown mechanism	Mouse	iPSCs	No	During reprograming, cells derived from WNT-expressing cells establish clone dominance	Shakiba *et al* (2019)
APC	Unknown mechanism	*Drosophila*	Intestine epithelium	Yes	Cells with loss of APC eliminate WT cells and cause growth of intestinal adenomas	Suijkerbuijk *et al* (2016)
APC	Unknown mechanism	Human and mouse	Intestinal 3D organoids; in vivo adenoma formation in mice	Yes	$APC^{-/-}$ intestinal stem cells secreted WNT antagonists that induced differentiation of nearby WT cells in organoids and resulted in tumor formation in mice.	Flanagan *et al* (2021), van Neerven *et al* (2021)
NOTCH	Unknown mechanism	Mouse	Esophageal epithelium	Yes	Cells with loss of NOTCH signaling outcompete their WT neighbors	Alcolea and Jones (2015)
TP53	Unknown mechanism	Mouse	Embryo (epiblast)	No	p53 represses mTOR which induces loser cell elimination	Bowling *et al* (2018)
TP53	Unknown mechanism	Mouse	Hematopoietic stem cell and progenitors (HSPCs)	No	In an irradiated niche, low-expressing p53 HSPCs outcompete high-expressing p53 HSPCs, which become senescent	Bondar and Medzhitov (2010)
TP53	Mechanical CC	*Drosophila*	Wing disk	No	p53 acts as a sensor for cells with less resistance to apoptosis by mechanical stresses in overcrowded tissue	Wagstaff *et al* (2016)
TP53	Unknown mechanism	Mouse	Irradiated epidermis and esophagus		Following radiation, epithelial Mutant p53 progenitors rapidly expand at the expense of wild-type p53 progenitors	Fernandez-Antoran *et al* (2019), Murai *et al* (2018)
mTOR	Unknown mechanism	Mouse	Embryo (epiblast)	No	p53 represses mTOR which induces loser cell elimination	Bowling *et al* (2018)
BMP	Similar to trophic factor	Mouse	Embryo (epiblast)	No	Defective BMP cells express lower MYC and are eliminated from the epiblast	Sancho *et al* (2013)
EGFR	Unknown mechanism	*Drosophila*	Imaginal disk	Yes	EGFR-overexpressing supercompetitors generate aggressive tumors and induce apoptosis in surrounding normal epithelium	Eichenlaub *et al* (2016)
JAK-STAT	Unknown mechanism	*Drosophila*	Imaginal disk and eye	No	Cells with hyperactive STAT signaling function as supercompetitors that induce apoptosis in wild-type cells	Rodrigues *et al* (2012)

List of cell competition genes identified, their mechanism, the organism/tissue/cell type employed to study, role in cancer, and summary of key findings. Representative findings for each gene have been included to briefly summarize.

short-range modes of molecular exchange of soluble signaling cues or membrane-bound fitness fingerprints (Levayer *et al*, 2016; Wagstaff *et al*, 2016; Moreno *et al*, 2019).

Activation of receptor-mediated cell death

This mode of CC proceeds through direct cell–cell contact in which a ligand–receptor system at the interface between competing cells drives elimination of losers or via ligands secreted by winner cells that directly activate cell death in loser cells. In *Drosophila*, this mode of CC can be observed in Scribble-deficient ($scrib^{-/-}$) epithelial cells that have loss of apico-basal polarity (Table 1). When $scrib^{-/-}$ (MT) cells come in contact with WT cells, the ligand stranded at second (Sas) on WT and the surface receptor PTP10D on MT cells re-localize laterally, resulting in transactivation of Sas-PTP10D signaling (Yamamoto *et al*, 2017). As an outcome, EGFR signaling is inactivated, while JNK pro-apoptotic pathway is induced

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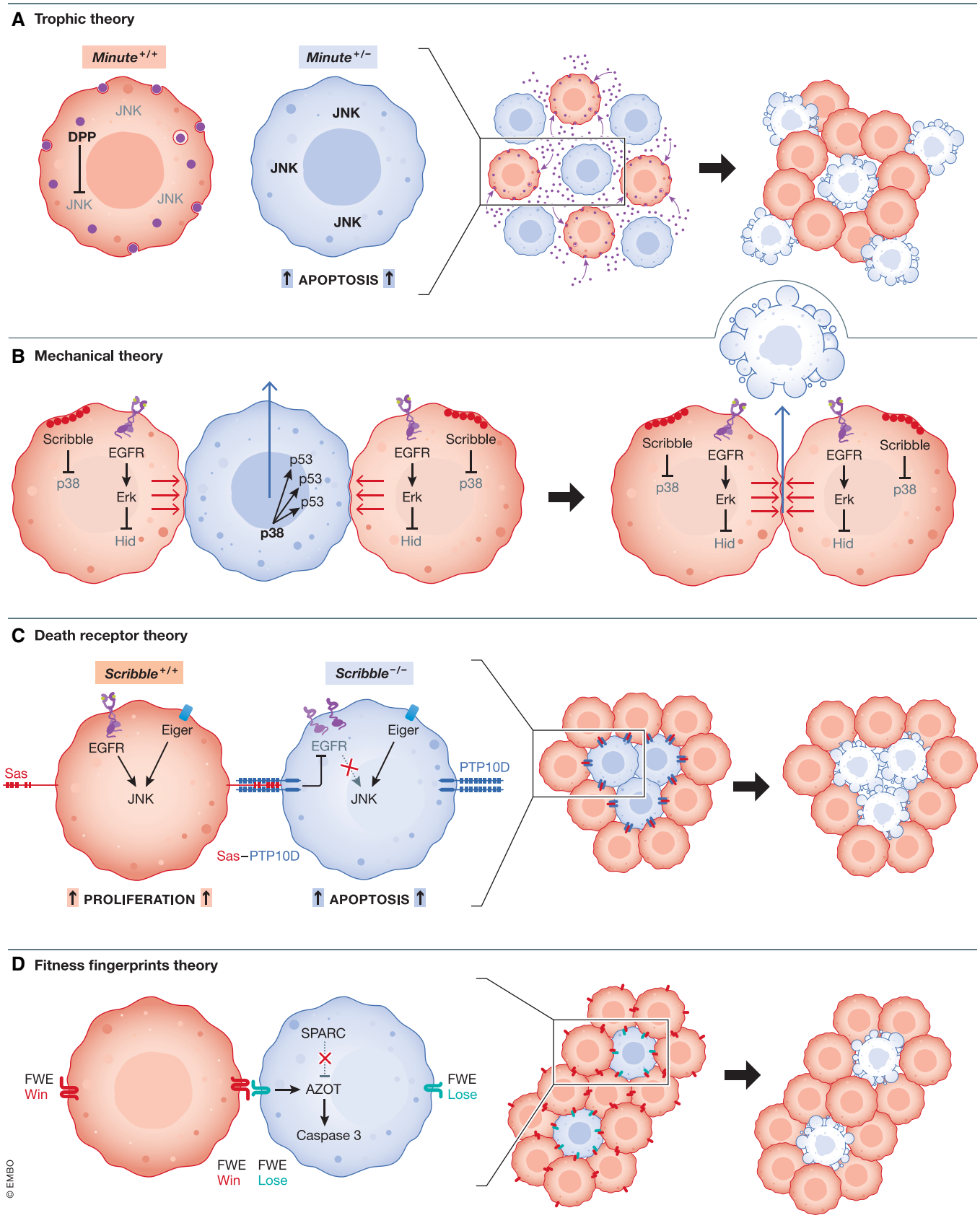

Figure 1.

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Figure 1. General mechanisms of cell competition.

(A) Model of indirect CC based on trophic theory: Growth-deficient ribosomal protein gene cells ($RP^{+/-}$) are outcompeted by wild-type cells ($Rp^{+/+}$) based on their reduced ability to capture trophic factors, such as Dpp, essential for their survival. Dpp allows cells to block the activation of apoptotic pathways and maintain a proliferative state. The lower capacity of growth-deficient $RP^{+/-}$ cells to obtain Dpp leads to the activation of JNK apoptotic signaling and ultimately their replacement by wild-type cells that efficiently capture Dpp ligands. (B) Model of Mechanical theory: Mechanical-based cell competition regulates cell growth and survival. Differential sensitivities to mechanical forces in epithelial tissues results in elimination of suboptimal cells by their healthy neighbors, and compensatory gain of winners to the tissue space. At the molecular level, in suboptimal cells, the high levels of stress and deformation downregulate ERK signaling and increase the expression of the apoptotic proteins p38, p53, and Hid. Suboptimal cells undergo apical extrusion, or apoptosis, and optimal cells expand. (C) Model of Death Receptor theory: Healthy cells can induce the elimination of suboptimal mutant neighbors by activation of cell surface ligand-death receptor pairs. *scribble* (*scrib*)$^{-/-}$ MT cells expressing PTP10D receptor contact WT epithelial cells expressing surface ligand, Sas inducing transactivation of Sas-PTP10D signaling and subsequent EGFR inactivation and JNK pro-apoptotic signaling in *scrib*$^{-/-}$ loser cells, and compensatory proliferation of WT cells. Eiger (TNF superfamily member) activates JNK-dependent cell death in *scribble* polarity mutants. (D) Model of cell competition via fitness fingerprints. Neighboring cells compare their fitness status through the expression of fitness fingerprints, such as transmembrane Flower proteins. Flower Win-expressing cells recognize Flower Lose-expressing suboptimal cells. Consequently, Flower Lose upregulates Azot, which induces caspase-dependent apoptosis. In some cases, suboptimal cells can increase the expression of SPARC to antagonize Flower Lose-mediated apoptosis and transiently prevent cell death.

in MT cells, thereby eliminating the polarity-defective transformed cells (Fig 1C). Though these cells express both death ligands and receptors at their membranes, the activation of death signals is only observed in loser cells (Yamamoto *et al*, 2017). Elimination of polarity-mutant losers in *Drosophila* epithelia was also shown to depend upon Eiger, the *Drosophila* tumor necrosis factor (TNF), and downstream JNK activation (Igaki *et al*, 2009).

Toll receptor signaling provides another compelling example by which winner cells eliminate adjacent loser cells by direct activation of cell death in losers. Current evidence suggests that this immunological process inhibits tissue growth and regulates cell fitness during infection (Germani *et al*, 2018). However, it is widely reported to be repurposed for non-infectious cell selection (Meyer *et al*, 2014). In *Drosophila* wing disks, forced Myc expression or *Minute*-induced competition in *RpL14*$^{-/+}$ cells leads to Toll-related receptors (TRRs) activation by the secreted factor Spätzle, leading to NF-kB mediated apoptosis (Meyer *et al*, 2014; Alpar *et al*, 2018). This behavior can also be mimicked in a cancer-like invasive phenotype in Tai-expressing *Drosophila* pupal wings, where its expression induces Spätzle-Toll-mediated apoptosis of loser cells and invasion by winner cells in the thoracic region (Byun *et al*, 2019). In contrast, in *scrib*$^{-/-}$ clones that are extruded (discussed later) from imaginal epithelia, TRR activation transforms these loser *scrib*$^{-/-}$ cells into winners by activation of Hippo signaling and prevention of cell death(Katsukawa *et al*, 2018) (Table 1).

Fitness fingerprints

Mechanistic detection of cellular fitness remains unclear. Thus far, the only fitness-sensing mechanism identified is the Flower (Fwe) "fitness fingerprints", which are highly conserved transmembrane proteins that directly communicate fitness status and drive competition (Table 1) (Rhiner *et al*, 2010; Madan *et al*, 2019b). In *Drosophila*, there are three alternately spliced Flower isoforms: Fwe^{ubi}, Fwe^{LoseA}, and Fwe^{LoseB}. The ubiquitous form, Fwe^{Ubi}, is constantly expressed. Under competitive stress, Fwe^{ubi} is downregulated and Fwe^{LoseA} and Fwe^{LoseB} are upregulated in loser cells that undergo apoptosis (Rhiner *et al*, 2010). Importantly, membrane $Flower^{Lose}$ presence does not autonomously induce apoptosis and $Flower^{Lose}$ cells are not eliminated when surrounded by $Flower^{Lose}$ neighbors (Rhiner *et al*, 2010). Secondly, $Flower^{Lose}$ cells can upregulate the expression of secreted protein acidic and cysteine-rich protein (SPARC), which inhibits caspase induction of apoptosis as a transient defense against elimination (Portela *et al*, 2010). Thus, SPARC may serve as defense against unnecessary purging, particularly as means to counteract supercompetition. In contrast, loser cell upregulation of the gene, *Azot,* especially in weakened cells with diminished SPARC, ensures competitive elimination by apoptosis (Merino *et al*, 2015) (Fig 1D). Interestingly, expression of $Flower^{Lose}$, Azot, and SPARC is impacted by various triggers of competition and supercompetition such as modulation of Dpp morphogen signaling or dMyc expression, and genetic mutation in genes encoding ribosomal proteins (Rp mutants) (Rhiner *et al*, 2010; Merino *et al*, 2015). This suggests the Flower code of fitness fingerprints and its downstream players are a common, tunable read-out of cellular fitness likely functional in many tissue systems as means to continuously survey cellular status and rid suboptimal cells.

Flower is a highly conserved gene, and this fitness-sensing program also occurs in mammals (Rhiner *et al*, 2010; Madan *et al*, 2019b). In humans, the *Fwe* locus generates four isoforms: two $hFwe^{Win}$ and two $hFwe^{Lose}$. Two Flower isoforms (hFWE2 and hFWE4) behave as Flower-Win proteins, whereas the other isoforms (hFWE1 and hFWE3) behave as Flower-Lose proteins. Like in *Drosophila*, hFwe-Lose-expressing cells are viable in a homotypic environment. However, when confronted by hFwe-Win-expressing cells, Lose cells are outcompeted and undergo apoptosis. Human Flower-mediated competition requires contact between Win and Lose cells and was not dependent on soluble signal exchange (Madan *et al*, 2019b) (Table 1).

Other mechanisms

Several additional gene pathways have been implicated in CC, but do not fit the mechanisms as described above. We do not yet understand how interacting cells sense the imbalance in these pathways or whether these pathways fit within a larger scheme of signaling events underlying CC. In general, differential expression or activity of the following gene pathways result in differences in growth, proliferation, polarity, and/or survival that generates competitive interactions between the neighboring cells. These include Myc (de la Cova *et al*, 2004; Claveria *et al*, 2013; Di Giacomo *et al*, 2017; Diaz-Diaz *et al*, 2017), Ras and Src (Kajita *et al*, 2010; Kajita *et al*, 2014), Scribble (Norman *et al*, 2012), Mahjong with Lgl (Tamori *et al*, 2010), TEAD and YAP/TAZ (Hashimoto & Sasaki, 2019; Moya *et al*, 2019), Wnt/Wg (Vincent *et al*, 2011; Suijkerbuijk *et al*, 2016; Akieda *et al*, 2019; Flanagan *et al*, 2021; van Neerven *et al*, 2021), Janus Kinase (JAK)-signal transducer and activator of transcription (STAT) (Rodrigues *et al*, 2012), Notch (Alcolea & Jones, 2015),

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EGFR (Eichenlaub *et al*, 2016; Moreno *et al*, 2019), mTOR (Bowling *et al*, 2018), and p53 (Bondar & Medzhitov, 2010; de la Cova *et al*, 2014; Bowling *et al*, 2018; Murai *et al*, 2018; Fernandez-Antoran *et al*, 2019) (Table 1). The most well studied example is CC induced by imbalances in the activity of Myc, a transcription factor that positively impacts proliferation and growth. High Myc-expressing cells act as supercompetitors and outcompete low Myc-expressing or wild-type cells (de la Cova *et al*, 2004; Moreno & Basler, 2004; Claveria *et al*, 2013; Di Giacomo *et al*, 2017; Diaz-Diaz *et al*, 2017). *Myc*-mediated competition appears to require direct contact, but one study noted *dMyc*-mediated competition across several cell diameters (de la Cova *et al*, 2004). Additionally, the role of molecule exchange or diffusible signal is under debate (Claveria *et al*, 2013; Sancho *et al*, 2013). Some evidence suggests *dMyc*-mediated competition is through competition for Dpp capture; however, growth cues appear to be an incomplete mechanism (de la Cova *et al*, 2004). Thus, there are still many missing pieces in how cells infer fitness based on *Myc* status as well as the other pathways listed. Extensive crosstalk exists between these pathways—i.e., Myc and YAP/TAZ have been shown to cooperate to promote CC-based elimination (Hashimoto & Sasaki, 2019), and p53 status has been identified as a sensor in various mechanisms of CC (de la Cova *et al*, 2014; Wagstaff *et al*, 2016; Bowling *et al*, 2018). Additionally, differential signaling in dMyc, WNT/Wg, and JAK-STAT instigates CC via Flower fitness sensing (Rhiner *et al*, 2010).

Cell competition between tumor and its microenvironment

Surveillance and clearance of abnormal or transformed cells is an absolutely essential process for tissue maintenance and tumor prevention. Given its role in the detection and culling of aberrant cells, CC has been proposed to serve as a brake to tumorigenesis. In this section, we will discuss the mechanisms by which CC prevents neoplastic tumor development. We will also discuss how failure of homeostatic CC within the tissue microenvironment releases the break and facilitates clonal expansion of transformed cells into subsequent frank carcinoma. Secondly, we discuss how in some contexts cancer cells hijack CC mechanisms and act as supercompetitors resulting in tumor outgrowth at the expense of normal tissue. Moreover, we frame CC mechanisms in the context of tumors as complex ecosystems to hypothesize how CC may regulate intratumoral CC, particularly under hypoxia and following therapy.

Cell competition suppresses tumorigenesis

For the majority of epithelial cancers, a tumor develops from a single transformed cell. How the transformed cell and its early clonal expansion evade regulatory features imposed by its tissue microenvironment is not well understood. However, tissues appear to harbor intrinsic tumor suppressive features to rid mutant, pretumoral cells. One process, epithelial defense against cancer (EDAC), is an active mechanism whereby WT cells sense and eliminate newly transformed cells (Kajita *et al*, 2010; Kajita *et al*, 2014; Kon *et al*, 2017). Importantly, this elimination of abnormal cells occurs only in the presence of normal cells and fails if an entire epithelium becomes comprised of mutants (Kon *et al*, 2017). This phenomenon is similar to the principles of CC. Using MDCK cells, a non-transformed mammalian epithelial cell line, it was shown that Src-activated or Rasv12-transformed cells underwent apoptosis-independent apical extrusion from the monolayer when co-cultured with WT cells (Hogan *et al*, 2009; Kajita *et al*, 2010; Kajita *et al*, 2014; Kon *et al*, 2017). MT cell extrusion is an active process driven by accumulation of the cytoskeletal proteins, filamin and vimentin, within normal cells at their interface with the mutants (Kajita *et al*, 2014) (Fig 2A). Simultaneously, dysfunctional cells display notable differences in height, morphology, and elasticity accompanied by altered expression of cytoskeletal proteins and mechanotransduction cues required to extrude the cell (Kajita *et al*, 2010; Kajita *et al*, 2014). Additionally, normal cells can alter the metabolic profile of dysfunctional or transformed cells to further promote their extrusion (Kon *et al*, 2017). The efficiency of live-cell extrusion to prevent tumorigenesis in different tissue types, including those without inherent clearance mechanisms (i.e., clearance of outcompeted cells with waste in the intestine), is for debate. On this note, clearance of outcompeted transformed cells may proceed through engulfment of live outcompeted cells or their corpses. For example, in *Drosophila* imaginal epithelia with *scrib* deletion, the WT cells were found to engulf the $scrib^{-/-}$ loser cells via non-apoptotic JNK signaling, demonstrating that engulfment may be another mechanism by which the neoplastic loser cells are eliminated (Ohsawa *et al*, 2011) (Fig 2A-b). Additionally, losers can undergo apoptosis prior to apical extrusion by active CC (Norman *et al*, 2012; Wagstaff *et al*, 2016). Polarity mutant cells generated by knockdown of *Scribble* ($scrib^{KD}$) are outcompeted by their WT neighbors and undergo p38-mediated apoptosis, followed by apical extrusion (Norman *et al*, 2012) (Fig 2C). $Scrib^{KD}$ cells in contact with WT neighbors were hypersensitive to mechanical stress from tissue overcrowding, due to their elevated baseline p53 signaling, which marked them as losers. Contact with WT cells compacted $scrib^{KD}$ into high-density arrangements, with subsequent activation of p38 stress signaling and further upregulation of p53, driving their apoptotic elimination and extrusion from the epithelial monolayer (Wagstaff *et al*, 2016) (Fig 2A-c).

These models of tumorigenesis using *Drosophila* epithelia had shown that the fate of mutant cells relies upon direct interactions between them and their normal neighbors. EDAC mechanisms have also been demonstrated using RasV12 mosaics within the mouse intestine, pancreas, and lung epithelia. Single RasV12 mutants confronted by an otherwise normal epithelium underwent apical extrusion, in support of similar RasV12 manipulations in vitro (Kon *et al*, 2017; Sasaki *et al*, 2018). MT cell extrusion blocked development of tumors. However, in a highly inflammatory microenvironment, which is a major driver of several malignancies, EDAC-based CC fails and RasV12 mutants are retained, resulting in tumors (Sasaki *et al*, 2018). Crosstalk between a tumor and its microenvironment was investigated in depth further by genetic manipulation of the CC factors YAP/TAZ using a mouse model of liver cancer (Moya *et al*, 2019). Upregulation of YAP/TAZ is frequently observed in human cancers (Zhou *et al*, 2016) and high YAP/TAZ has previously been shown to generate supercompetitors (Hashimoto & Sasaki, 2019). Mouse liver tumors harboring high levels of active YAP/TAZ were generated and predictably grew at the expense of peritumoral tissue, which showed signs of apoptosis. However, when YAP was hyper-activated in peritumoral normal hepatocytes, tumor growth was not only strongly suppressed, but tumor cells

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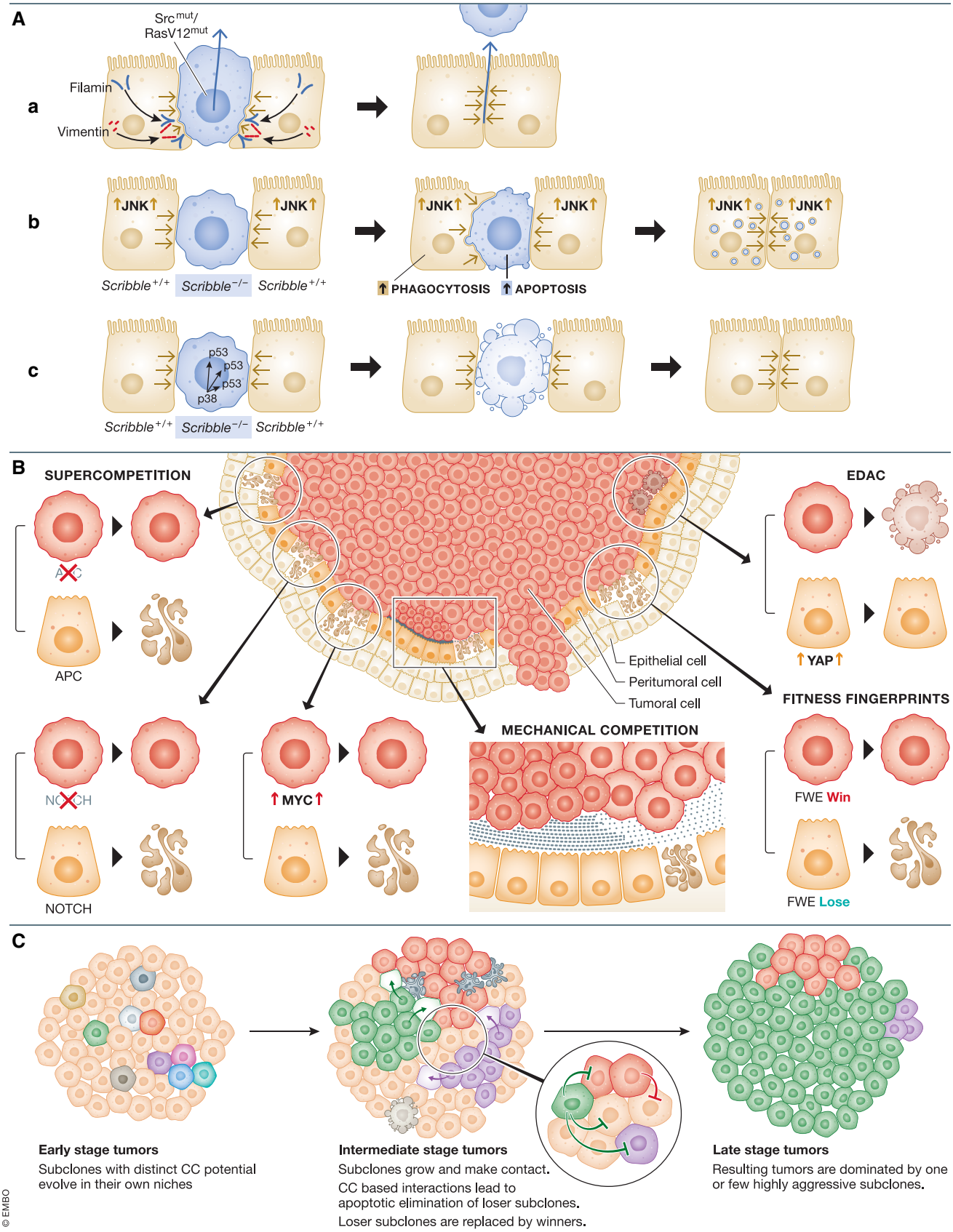

Figure 2.

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Figure 2. Cell competition between newly transformed cells and normal neighbors.

(A) (a). Epithelial defense against cancer (EDAC): a) EDAC supports removal of newly transformed cells with activated RasV12 or Src mutations by neighboring normal cells. Normal cells upregulate filamin and vimentin at border of transformed cell, inducing pressure on transformed cell. RasV12 or Src mutants undergo live-cell apical extrusion. (b) Polarity-defective *Scribble*$^{-/-}$ mutants undergo JNK-mediated apoptosis in presence of wild-type (WT) cells. Concurrently, WT cells activate JNK signaling resulting in activation of phagocytic pathways. WT cells engulf apoptotic *Scribble*$^{-/-}$ mutants, eliminating them from the tissue space. (c) In a separate mechanism, the presence of WT cells induces activation of p38 and p53 apoptotic signaling in *Scribble*$^{-/-}$ mutants, resulting in their apoptotic elimination from epithelia. (B) Cell competition between tumor and adjacent stromal cells. Tumor cells take advantage of competition mechanisms to promote their survival and eliminate stromal cells. Loss of APC and NOTCH or upregulation of fitness-enhancing genes such as MYC in tumor cells generates supercompetitors that outcompete their wild-type neighbors. Tumor cells expressing the fitness fingerprint Flower Win (Fwe-Win) can sense and eliminate via apoptosis Fwe-Lose-expressing stromal cells, resulting in tumor outgrowth at the expense of normal tissue. Increased activation of YAP in peritumoral epithelial cells (red epithelial cells) leads to the apoptosis of tumor cells in a mode similar to EDAC to suppress tumor outgrowth. RasV12 mutants act as supercompetitors and outcompete surrounding normal cells via mechanical compression-induced apoptosis. This can potentially facilitate the formation of tumors. (C) Tumor evolution based on cell competition principles: Clones evolve through advantageous driver mutations and competition mechanisms impact clonal dominance. During the early stages of tumor growth, distinct clones develop within their unique niches selected for by the microenvironment. As subclones engage in various forms of competition, this results in expansion of winner clones at the expense of loser clones (represented by empty cell-outlines of dead loser cells being replaced by neighboring winner cells marked by arrows). A late stage tumor is dominated by select highly aggressive subclones that have outcompeted losers through rounds of cell competition.

enclosed by YAP-activated hepatocytes were apoptotic (Moya *et al*, 2019) (Fig 2B). These results suggest that supercompetitor normal cells can overtake oncogenic growth mechanisms of tumor cells and that a tissue microenvironment with high fitness has the capacity to restrain tumor growth.

Cell competition promotes mutant clonal expansion and tumor formation

Cancer cells are characterized by uncontrolled cellular division as they invade into the surrounding stroma. One possible mechanism by which the tumor manipulates its microenvironment to accomplish invasion is to exploit CC to create space by outcompeting and eliminating the non-malignant neighbors. This phenomenon is particularly interesting given recent findings that CC can occur between different cell types, i.e., between epithelial cells and fibroblasts, which are a major cell type in the stroma of various tissues (Madan *et al*, 2019b). Cells with a supercompetitor status generate clones capable of expansion into fields of mutant cells, at the expense of normal cells, often with no changes in tissue morphology. This is very similar to a process known as field cancerization in which mutant clones expand into fields, at the expense of normal cells, often with no accompanied changes in tissue morphology. Overtime, these fields can develop into malignant tumors assuming additional accumulation of genetic mutations (Braakhuis *et al*, 2003). In a mechanism independent of fitness sensing or selection, oncogene-activated cells exploited mechanical compression as a form of supercompetition to cause crowding-induced cell death randomly in normal cells. In the *Drosophila* wing disk, oncogenic Ras clones rapidly expanded and sustained high growth despite compressive stress due to their resistance to apoptosis. To alleviate this stress and create space for their own growth, Ras MT clones instead compressed and activated apoptosis in neighboring WT cells, even up to several cell diameters away from the MT clonal boundaries (Levayer *et al*, 2016). Supercompetition resulting in field cancerization, or the vast expansion of MT clones, has also been shown in mammalian systems such as the normal mouse esophageal epithelium wherein loss of Notch signaling generates MT winners that rapidly expand and drives adjacent WT progenitors to differentiate (Alcolea & Jones, 2015) (Fig 2B) (Table 1). Similarly, UV-exposure in the normal mouse epidermis (Murai *et al*, 2018), and ionizing radiation (IR) in the normal mouse esophageal epithelium drove clonal expansion of progenitors with GOF p53 mutations that outcompete and proliferate at the expense of their WT neighbors that terminally differentiate and slough off (Murai *et al*, 2018; Fernandez-Antoran *et al*, 2019). These studies highlight how various stressors act as selective pressures that shift selection away from WT cells and skew toward MT clones. This may lower tissue fitness overtime, decrease EDAC-like CC strength, and result in malignancy.

Many genes shown to generate supercompetitors are known tumor suppressors or oncogenes: Myc (Claveria *et al*, 2013; Di Giacomo *et al*, 2017; Diaz-Diaz *et al*, 2017), JAK-STAT (Rodrigues *et al*, 2012), Notch (Alcolea & Jones, 2015), YAP/TAZ (Hashimoto & Sasaki, 2019; Liu *et al*, 2019b; Moya *et al*, 2019), Ras (Moreno *et al*, 2019), APC (Flanagan *et al*, 2021; van Neerven *et al*, 2021; Yum *et al*, 2021) and p53 (Murai *et al*, 2018; Fernandez-Antoran *et al*, 2019). Tumor cells behave as supercompetitors due to their growth, proliferation, and survival advantages. This was shown experimentally using tumorigenesis models in *Drosophila* epithelia by generating supercompetitors that were EGFR-overexpressing (Eichenlaub *et al*, 2016), or that were hyperactive in Wnt signaling with increased YAP expression due to deletion in the tumor suppressor gene, *APC* (Suijkerbuijk *et al*, 2016) (Fig 2B). In both scenarios, mutant supercompetitors generated aggressive tumors, and tumor growth depended on apoptotic elimination of surrounding WT neighbors (Eichenlaub *et al*, 2016; Suijkerbuijk *et al*, 2016). EGFR-overexpressing winner cells engulfed losers and resulted in tumor cells with additional polyploidization and faulty epithelial polarity. These tumor cells were more aggressive with enhanced metastatic capacity (Eichenlaub *et al*, 2016). Thus, engulfment may provide nutrients and metabolites to winners to sustain their high growth and proliferation demands. It is noteworthy that signs of entosis, or the engulfment of live cells, have been observed in human tumors (Sun, Luo *et al*, 2014).

APC$^{-/-}$-driven supercompetition was recently uncovered as an active mechanism in human intestinal stem cells to achieve mutant clonal expansion (Flanagan *et al*, 2021; van Neerven *et al*, 2021). Notably, the *APC* tumor suppressor gene is mutated in nearly 80% of human colon cancers (van Neerven *et al*, 2021). In corroboration with findings from *Drosophila* (Suijkerbuijk *et al*, 2016), *APC*$^{-/-}$ mutant intestinal stem cells derived from both mouse and human tissue acted as supercompetitors and outcompeted WT neighbors in 3D organoid cultures (van Neerven *et al*, 2021). *APC*$^{-/-}$ supercompetitors secreted WNT antagonists that decreased growth and induced differentiation of adjacent WT stem cells (as opposed to apoptosis in *Drosophila*). This supercompetition resulted in large

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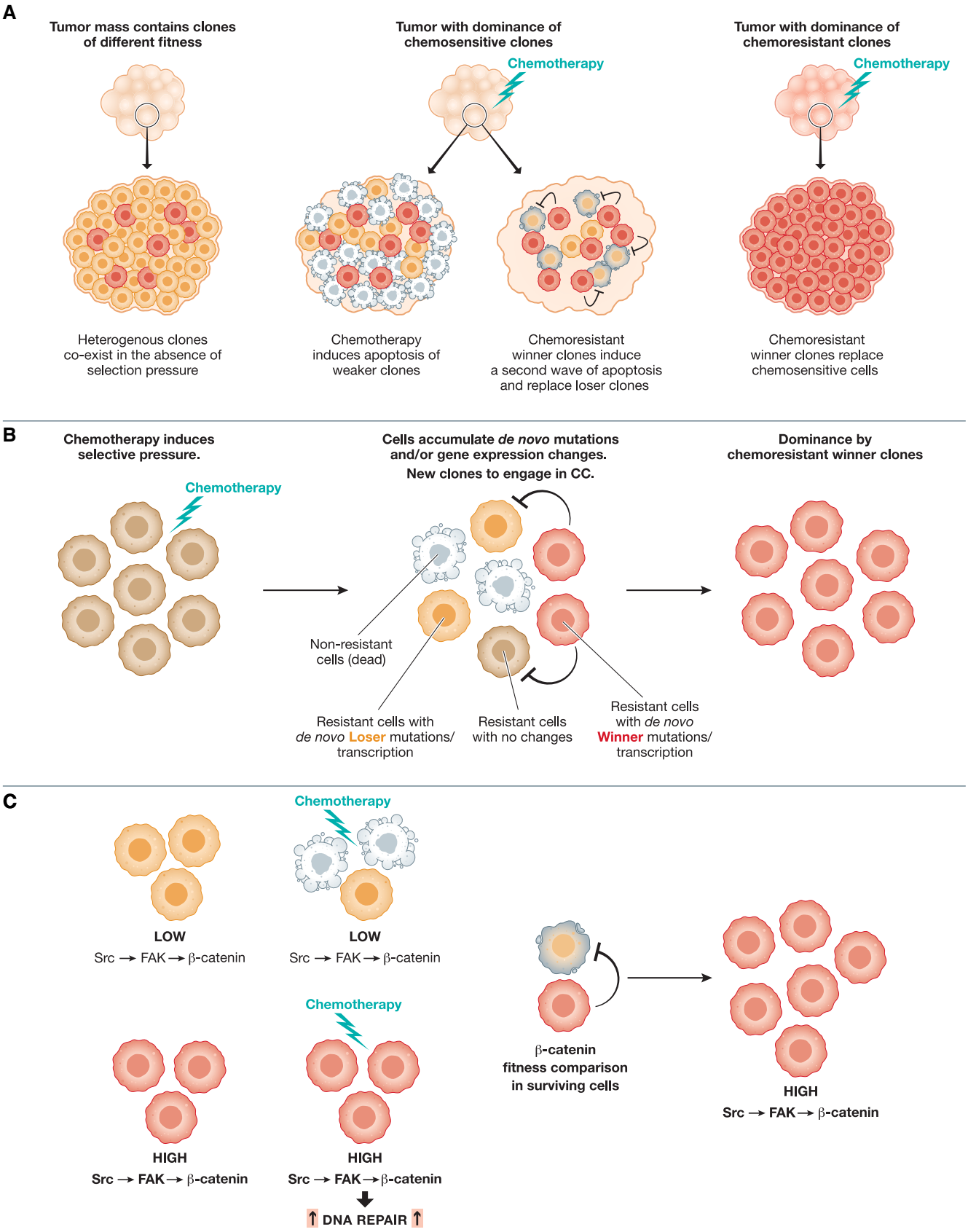

Figure 3.

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Figure 3. Cell competition in resistance to chemotherapy.

(A) Tumors harbor therapy-resistant subclones. Chemotherapy results in massive cell death, which frees space for resistant clones to grow. Resistant cells now engage in competition-based elimination of less-fit cells and generate a more aggressive, "superfit" tumor. (B) Chemotherapy regimens induce large-scale mutations and gene-expression changes that can eliminate most non-chemotherapy-resistant cells. However, in a small population of surviving chemotherapy-resistant cells, de-novo mutations and gene-expression changes pertinent to cell competition occur as a result of chemotherapy-induced selection pressure. These distinct populations can now engage in cell competition, and a single population is expected to be clonally dominant in the relapsed tumor. (C) An example of selection of a chemoresistant population with greater fitness. Src-induced FAK/β-catenin activity results in chemotherapy resistance. Upon chemotherapy, chemosensitive cells are eliminated and remaining cells may undergo cell competition based on β-catenin activity, in which cells with higher β-catenin activity function as winners. This ultimately would generate a tumor with strong chemotherapy resistance.

intestinal adenomas in $Apc^{-/-}$ mice, but outcompetition was negated upon disruption of WT stem cell sensitivity to secreted WNT antagonists via pharmacologic or genetic intervention (Flanagan *et al*, 2021; van Neerven *et al*, 2021). Findings from these studies highlight a conserved mechanism of CC between mutant and wild-type cells that dictates clonal selection and fuels tumorigenesis. In further support, intestinal stem cells carrying common colon cancer-associated mutations in either *Kras* or *Pik3ca* were also shown to outcompete their surrounding normal neighbors in mouse intestinal crypts. MT *Kras* or *Pik3ca* crypts secreted BMP ligands that restrained stem cells and increased differentiation rates in WT crypts. MT crypts further manipulated their environments by inducing normally supportive proximal stromal cells to instead secret WNT inhibitors that additionally drove stem cell differentiation. Importantly, the MT cells were not as vulnerable to WNT inhibitors and maintained high turnover, cementing the competitive advantages of MT clones over WT neighbors (Yum *et al*, 2021). Thus, CC appears to underlie tissue remodeling efforts by MT cells to facilitate expansion and possibly, eventual tumor progression.

Additional data from human cancers suggest that supercompetitive Myc-high tumor cells change the tumor microenvironment by inducing apoptosis in their neighboring stromal cells with relatively lower Myc expression (Di Giacomo *et al*, 2017). *Myc*-overexpressing cells outcompete and eliminate low-*Myc* cells via apoptosis (Moreno & Basler, 2004; Claveria *et al*, 2013). Human cancers bear a striking resemblance to this mechanism as cancer cells frequently express higher levels of c-Myc compared to stromal cells, which correlates with increased cleaved caspase in the stroma (Di Giacomo *et al*, 2017) (Fig 2B). There is additional evidence that tumor cells can manipulate stromal cell fate by taking advantage of CC fitness fingerprint mechanisms (Madan *et al*, 2019b). Various human cancers were shown to express high levels of $hFwe^{Win}$ isoforms in the tumor tissue and $hFwe^{Lose}$ isoforms in the stroma, which displayed signs of apoptosis (Madan *et al*, 2019b) (Fig 2B). *In vivo* testing of fitness fingerprints in tumorigenesis by implantation of $hFwe^{Win}$-expressing human breast cancer cells into $hFwe^{Lose}$-expressing mammary tissue in mice generated large, aggressive tumors. In contrast, implantation of $hFwe^{Lose}$ cells into a $hFwe^{Win}$ tissue background failed to develop into tumors. These findings align with those obtained from YAP/TAZ mediated CC in liver cancer (Moya *et al*, 2019), supporting that tissue fitness and CC between the tumor and the surrounding microenvironment directly impacts tumorigenesis. Additionally, the stroma of many cancers shows high expression of SPARC, a protein upregulated in loser cells in response to CC, including in Flower-fitness mechanisms (Petrova *et al*, 2011). This further indicates active CC occurs between confronted cancer and normal cells, and outcomes of this competition may very well alter the fate of the cancer.

Tissue architecture in the tumor microenvironment presents opportunities of mechanisms involving or resembling mechanical CC to affect oncogenic development (Fiore *et al*, 2020). One characteristic of aggressive cancers is their ability to grow uncontrollably and invade neighboring tissues, which is only possible when tumors overcome spatial constraints, potentially due to mechanical CC between tumors and their surrounding tissue. As an example, basal cell carcinomas (BCCs), a slow growing, typically non-metastatic cancer (Crowson, 2006), grow in "buds", possibly due to softening and remodeling of the basement membrane, whereas invasive squamous cell carcinomas (SCCs) grow in bidirectional "folds", possibly due to membrane stiffening. Thus, the differential biophysical and biomechanical properties of the basement membrane that is positioned directly below the layer of basal epidermal progenitors that give rise to BCCs and SCCs can act differently in these cancers resulting in different architectures (Fiore *et al*, 2020) (Fig 2B). These different mechanical forces pervasive throughout the stratified epidermis act on the tumor cells and impact the invasive potential of epidermal progenitors at the tumor-stroma border.

Intratumoral competition

Tumors are heterogeneous masses containing distinct cancer and non-cancer cells that must compete for space and resources under the selective pressures exerted by their environments (Lu *et al*, 2012). While early tumors consist of relatively homogenous cell populations, intrinsic genomic instability of cancer cells along with various microenvironment and "host" defense factors introduce widespread heterogeneity of cancer clones (Greaves & Maley, 2012). In heterogeneous tumors, after an initial phase of uniform growth of various subclones, a phase of rapid expansion follows where one or two clones emerge at the cost of others, indicating that the selection of dominant subclones is a time-dependent process (Gao *et al*, 2016). We hypothesize that CC between tumor cells results in the selection of the distinct clones that harbor distinct fitness advantages (Fig 2C). For example, human tumors show heterogeneous expression of Myc (Gupta *et al*, 2017) and human cancer cells with high c-Myc outcompete cancer cells with low c-Myc (Patel *et al*, 2017). Similarly, it was recently shown that YAP protein expression is highly heterogeneous in glioblastoma. Differential YAP expression between glioma cells results in clonal dominance of high-YAP cells and apoptosis of low-YAP cells and an overall more aggressive tumor (Liu *et al*, 2019b). These findings directly show how CC between different clones can potentially exacerbate oncogenic growth by dominance of more competitive clones.

The primary intrinsic driver of intratumoral heterogeneity (ITH) is the stepwise accumulation of somatic mutations in parental clones, followed by subclonal branching, a term that defines clones

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with additional mutations that are different from the parental clone (Nowell, 1976). ITH also results from the various stromal cell types including immune cells as well as the extracellular matrix (Ramon *et al*, 2020). Next generation sequencing technologies and mathematical modeling have shown that principles of population genetics also apply to the evolving tumor, whereby multiple clones may emerge from an initial genetic event, similar to the allelic variation in a population (Hu *et al*, 2017). Therefore, neutral drift and stochastic processes (Mao & Wheldon, 1995; Ling *et al*, 2015; Williams *et al*, 2016)-terms that define the gradual emergence of new variants in a population and may cause new clones to emerge, some of which have altered CC potential. This is in contrast with a Darwinian model of tumor evolution where mutations that are beneficial to the tumor are selected for and are often a result of selection bottlenecks such as chemotherapy, hypoxia and other microenvironment factors (Gerlinger & Swanton, 2010; Polyak, 2014; Lloyd *et al*, 2016). Regardless of the genetic principles that lead to the emergence of new clones, the dominance of the resulting tumor by cells bearing winner mutations or gene-expression changes suggests that CC-based selection of clones occurs during tumor evolution.

The variable access to space and nutrients by cancer clones introduces competition between subclones based on spatial partitioning of the clone within the tumor. In addition, tumor cells must adapt to selective pressures such as hypoxia, and chemotherapy (Ramon *et al*, 2020). We have previously speculated that many of these hypoxia-induced metabolic and transcriptional changes that result in gain of aggressive phenotype have potential to crosstalk with CC pathways and thus fuel clonal evolution and heterogeneity (Madan *et al*, 2020). In one example of crosstalk, hypoxic zones frequently harbor mutant p53 clones that boost tumor aggressiveness (Madan *et al*, 2019a), meanwhile MT p53 status has been associated with a winner phenotype in various epithelia (Murai *et al*, 2018; Fernandez-Antoran *et al*, 2019). Possibly, hypoxic MTp53 clones act as winners that outcompete WTp53 clones, further manipulating clonal evolution and tumor aggression.

In the following subsection, we discuss potential CC pathways that may be activated as a result of chemotherapy and confer winner status to cancer clones that successfully activate these pathways. We propose CC mechanisms play an essential role in clonal selection by constantly sensing and replacing clones not conducive to thrive in the harsh environments generated from therapy.

Cell competition and cancer therapy

Therapy is an artificial selection pressure that acts on tumor cells and can lead to the expansion of rare clones due to intrinsic or adaptive-resistant features (Diaz *et al*, 2012). Several studies have shown that cells within the relapse-driven clone are often present as a low-frequency subclone prior to treatment, indicating these subclones are selected during therapy (Diaz *et al*, 2012; Morrissy *et al*, 2016). Resistance to ibrutinib, an inhibitor of the Bruton's tyrosine kinase (BTK), is a compelling example of this where whole-exome sequencing and kinetic analysis identified that ibrutinib therapy led to the expansion of clones with mutations in TRAIL-R that were initially present in low numbers, favoring the pathways which are similar to the receptor-mediated cell death mechanism of CC in winner cells (Burger *et al*, 2016). More generally, platinum agent-based chemotherapy provides another instance of this phenomenon. Platinum agents are used in a wide number of cancers in combination with other chemotherapeutic drugs (e.g., FOLFOX in the colon and pancreatic cancer) (Grothey & Venook, 2018) or alone (e.g., in ovarian cancer) (Ledermann *et al*, 2018) to induce DNA damage, thus leading to cell apoptosis (Diaz Osterman *et al*, 2019) (Fig 3A). Secondly, these agents are genotoxic, and thus, cells surviving treatment are likely to accumulate additional mutations enabling them to regenerate a more aggressive tumor (Fig 3B). Therefore, mechanisms underlying therapy resistance and growth of clones following treatment-induced selective sweeps must be understood and accounted for in therapy rationale.

Studies have reported that focal adhesion kinase (FAK) activity leads to platinum resistance (Diaz Osterman *et al*, 2019). FAK is fully activated by forming a signaling complex with SRC (Brunton *et al*, 2005) and promotes nuclear accumulation of β-catenin (Chen *et al*, 2012), which is associated with CC mechanisms (Enomoto & Igaki, 2013; Akieda *et al*, 2019). Upon platinum therapy, subclones harboring higher activity of FAK have higher activity of SRC (Mitra & Schlaepfer, 2006) and increased β-catenin transcriptional activity (Diaz Osterman *et al*, 2019) (Fan *et al*, 2019) (Fig 3C). Similarly, subclones with GOF mutations in genes such as *KRAS*, *NRAS* and *BRAF* have constitutive activation of EGFR/RAS/MAPK pathway, and can bypass EGFR blockade and are thus resistant to cetuximab therapy (anti-EGFR therapy) (Lievre *et al*, 2006). Additionally, enhanced EGFR/RAS/MAPK signaling confers a competitive advantage (Moreno *et al*, 2019) and would enable these clones to outcompete their surrounding WT neighbors, ultimately resulting in a more aggressive, and therapeutically resistant tumor. Similarly, increased nuclear translocation of the CC effector, YAP, which can also be induced by hypoxia, leads to chemoresistance (Dai *et al*, 2016) again highlighting how CC pathways potentially converge with molecular mechanisms that drive enhanced oncogenic properties of tumors such as hypoxia and drug resistance. Chemotherapy eliminates sensitive cells leaving behind clones with intrinsic resistance, or those that adapted to survive. We hypothesize that these alterations confer resistant cells different competitive status. Thus, CC mechanisms can be involved in parallel with chemotherapy, by selecting winner resistant clones that will establish their dominance over loser resistant clones. Lastly, it would be of interest to better predict the effects of therapy on competition and design therapeutic strategies that harness CC mechanisms to shift outcomes in favor of normal cells, and not repopulation of resistant oncogenic clones. In vitro modeling showed that treatment of normal human squamous cells with antioxidant or growth factors enhanced their fitness and they obtained the ability to outcompete Barrett's esophagus cells (Merlo *et al*, 2011). Similarly, antioxidant loading prior to irradiation of the mouse esophagus thwarted outcompetition of normal cells by MT-p53 progenitors and instead promoted normal cell proliferation (Fernandez-Antoran *et al*, 2019). Moreover, disruption of supercompetitor oncogenic mutant clonal expansion and tumor growth was achieved by reversion of loser status of surrounding WT neighbors through the disabling of competition by secreted factors (Flanagan *et al*, 2021; van Neerven *et al*, 2021). In summary, we propose that cancer therapy could potentially provoke unchecked CC and generate more aggressive tumors. However, there is potential to tilt the fate of CC by manipulation of the microenvironment and herein lies the power of CC that we argue should be taken into account in therapeutic rationale.

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Summary

In the current review, we discuss the classical and novel pathways involved in cell competition and their roles in cancer. Many CC genes such as *TP53*, *Myc*, Hippo signaling components *YAP/TAZ*, and others are repurposed by cancers to attain growth and survival advantages. These models elucidate key pathways such as cancer growth (Di Giacomo *et al*, 2017), cell death (Moreno *et al*, 2002), and loss of polarity (Merino *et al*, 2015) in CC-driven oncogenesis. Intratumoral competition is an inevitable facet of tumor biology, and results in enhanced tumor aggressiveness and complicates therapy. But lessons learned from CC studies may provide important insights into how cancer cells compete with each other and normal cells to overtake tissue space, particularly under the pretense of various microenvironment pressures. Recent studies presented in this review led us to postulate that tumor heterogeneity, and chemotherapy manipulate CC mechanisms to select for mutations that confer a supercompetitor phenotype and result in a more aggressive tumor. A better understanding of CC directly in drug resistance, which plague clinical interventions, is therefore highly important to better our understanding of cancer biology. But first, many fundamental questions in CC remain, such as fitness recognition mechanisms, alteration of cell fitness, and more comprehensive studies of how these homeostatic mechanisms are altered during cancer initiation and growth.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Akieda Y, Ogamino S, Furuie H, Ishitani S, Akiyoshi R, Nogami J, Masuda T, Shimizu N, Ohkawa Y, Ishitani T (2019) Cell competition corrects noisy Wnt morphogen gradients to achieve robust patterning in the zebrafish embryo. *Nat Commun* 10: 4710

Alcolea MP, Jones PH (2015) Cell competition: winning out by losing notch. *Cell Cycle* 14: 9 – 17

Alpar L, Bergantinos C, Johnston LA (2018) Spatially restricted regulation of spatzle/toll signaling during cell competition. *Dev Cell* 46: 706 – 719 e5

Bondar T, Medzhitov R (2010) p53-mediated hematopoietic stem and progenitor cell competition. *Cell Stem Cell* 6: 309 – 322

Bowling S, Di Gregorio A, Sancho M, Pozzi S, Aarts M, Signore M, D. Schneider M, Martinez-Barbera JP, Gil J, Rodríguez TA (2018) P53 and mTOR signalling determine fitness selection through cell competition during early mouse embryonic development. *Nat Commun* 9: 1763

Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH (2003) A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 63: 1727 – 1730

Brunton VG, Avizienyte E, Fincham VJ, Serrels B, Metcalf 3rd CA, Sawyer TK, Frame MC (2005) Identification of Src-specific phosphorylation site on focal adhesion kinase: dissection of the role of Src SH2 and catalytic functions and their consequences for tumor cell behavior. *Cancer Res* 65: 1335 – 1342

Burger JA, Landau DA, Taylor-Weiner A, Bozic I, Zhang H, Sarosiek K, Wang L, Stewart C, Fan J, Hoellenriegel J *et al* (2016) Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. *Nat Commun* 7: 11589

Byun PK, Zhang C, Yao B, Wardwell-Ozgo J, Terry D, Jin P, Moberg K (2019) The Taiman transcriptional coactivator engages toll signals to promote apoptosis and intertissue invasion in *Drosophila*. *Curr Biol* 29: 2790 – 2800.e4

Chen XL, Nam JO, Jean C, Lawson C, Walsh CT, Goka E, Lim ST, Tomar A, Tancioni I, Uryu S *et al* (2012) VEGF-induced vascular permeability is mediated by FAK. *Dev Cell* 22: 146 – 157

Claveria C, Giovinazzo G, Sierra R, Torres M (2013) Myc-driven endogenous cell competition in the early mammalian embryo. *Nature* 500: 39 – 44

de la Cova C, Abril M, Bellosta P, Gallant P, Johnston LA (2004) *Drosophila* Myc regulates organ size by inducing cell competition. *Cell* 117: 107 – 116

de la Cova C, Senoo-Matsuda N, Ziosi M, Wu DC, Bellosta P, Quinzii CM, Johnston LA (2014) Supercompetitor status of *Drosophila* Myc cells requires p53 as a fitness sensor to reprogram metabolism and promote viability. *Cell Metab* 19: 470 – 483

Crowson AN (2006) Basal cell carcinoma: biology, morphology and clinical implications. *Mod Pathol* 19: S127 – S147

Dai XY, Zhuang LH, Wang DD, Zhou TY, Chang LL, Gai RH, Zhu DF, Yang B, Zhu H, He QJ (2016) Nuclear translocation and activation of YAP by hypoxia contributes to the chemoresistance of SN38 in hepatocellular carcinoma cells. *Oncotarget* 7: 6933 – 6947

Di Giacomo S, Sollazzo M, de Biase D, Ragazzi M, Bellosta P, Pession A, Grifoni D (2017) Human cancer cells signal their competitive fitness through MYC activity. *Sci Rep* 7: 12568

Diaz Jr LA, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, Allen B, Bozic I, Reiter JG, Nowak MA *et al* (2012) The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 486: 537 – 540

Diaz Osterman CJ, Ozmadenci D, Kleinschmidt EG, Taylor KN, Barrie AM, Jiang S, Bean LM, Sulzmaier FJ, Jean C, Tancioni I *et al* (2019) FAK activity sustains intrinsic and acquired ovarian cancer resistance to platinum chemotherapy. *Elife* 8: e47327

Diaz-Diaz C, Fernandez de Manuel L, Jimenez-Carretero D, Montoya MC, Claveria C, Torres M (2017) Pluripotency surveillance by Myc-driven competitive elimination of differentiating cells. *Dev Cell* 42: 585 – 599.e4

Eichenlaub T, Cohen SM, Herranz H (2016) Cell competition drives the formation of metastatic tumors in a *Drosophila* model of epithelial tumor formation. *Curr Biol* 26: 419 – 427

Enomoto M, Igaki T (2013) Src controls tumorigenesis via JNK-dependent regulation of the Hippo pathway in *Drosophila*. *EMBO Rep* 14: 65 – 72

Fan Z, Duan J, Wang L, Xiao S, Li L, Yan X, Yao W, Wu L, Zhang S, Zhang Y *et al* (2019) PTK2 promotes cancer stem cell traits in hepatocellular carcinoma by activating Wnt/beta-catenin signaling. *Cancer Lett* 450: 132 – 143

Fernandez-Antoran D, Piedrafita G, Murai K, Ong SH, Herms A, Frezza C, Jones PH (2019) Outcompeting p53-mutant cells in the normal esophagus by redox manipulation. *Cell Stem Cell* 25: 329 – 341.e6

Fiore VF, Krajnc M, Quiroz FG, Levorse J, Pasolli HA, Shvartsman SY, Fuchs E (2020) Mechanics of a multilayer epithelium instruct tumour architecture and function. *Nature* 585: 433 – 439

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Flanagan DJ, Pentinmikko N, Luopajärvi K, Willis NJ, Gilroy K, Raven AP, Mcgarry L, Englund JI, Webb AT, Scharaw S *et al* (2021) NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. *Nature* 594: 430 – 435

Gao R, Davis A, McDonald TO, Sei E, Shi X, Wang Y, Tsai PC, Casasent A, Waters J, Zhang H *et al* (2016) Punctuated copy number evolution and clonal stasis in triple-negative breast cancer. *Nat Genet* 48: 1119 – 1130

Gerlinger M, Swanton C (2010) How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. *Br J Cancer* 103: 1139 – 1143

Germani F, Hain D, Sternlicht D, Moreno E, Basler K (2018) The Toll pathway inhibits tissue growth and regulates cell fitness in an infection-dependent manner. *Elife* 7: e39939

Greaves M, Maley CC (2012) Clonal evolution in cancer. *Nature* 481: 306 – 313

Grothey A, Venook AP (2018) Optimizing adjuvant therapy for localized colon cancer and treatment selection in advanced colorectal cancer. *J Natl Compr Canc Netw* 16: 611 – 615

Gupta N, Jung K, Wu C, Alshareef A, Alqahtani H, Damaraju S, Mackey JR, Ghosh S, Sabri S, Abdulkarim BS *et al* (2017) High Myc expression and transcription activity underlies intra-tumoral heterogeneity in triple-negative breast cancer. *Oncotarget* 8: 28101 – 28115

Hashimoto M, Sasaki H (2019) Epiblast formation by TEAD-YAP-dependent expression of pluripotency factors and competitive elimination of unspecified cells. *Dev Cell* 50: 139 – 154.e5

Hogan C, Dupre-Crochet S, Norman M, Kajita M, Zimmermann C, Pelling AE, Piddini E, Baena-Lopez LA, Vincent JP, Itoh Y *et al* (2009) Characterization of the interface between normal and transformed epithelial cells. *Nat Cell Biol* 11: 460 – 467

Hu Z, Sun R, Curtis C (2017) A population genetics perspective on the determinants of intra-tumor heterogeneity. *Biochim Biophys Acta Rev Cancer* 1867: 109 – 126

Igaki T, Pastor-Pareja JC, Aonuma H, Miura M, Xu T (2009) Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in *Drosophila*. *Dev Cell* 16: 458 – 465

Kajita M, Hogan C, Harris AR, Dupre-Crochet S, Itasaki N, Kawakami K, Charras G, Tada M, Fujita Y (2010) Interaction with surrounding normal epithelial cells influences signalling pathways and behaviour of Src-transformed cells. *J Cell Sci* 123: 171 – 180

Kajita M, Sugimura K, Ohoka A, Burden J, Suganuma H, Ikegawa M, Shimada T, Kitamura T, Shindoh M, Ishikawa S *et al* (2014) Filamin acts as a key regulator in epithelial defence against transformed cells. *Nat Commun* 5: 4428

Katsukawa M, Ohsawa S, Zhang L, Yan Y, Igaki T (2018) Serpin facilitates tumor-suppressive cell competition by blocking toll-mediated Yki activation in *Drosophila*. *Curr Biol* 28: 1756 – 1767.e6

Kon S, Ishibashi K, Katoh H, Kitamoto S, Shirai T, Tanaka S, Kajita M, Ishikawa S, Yamauchi H, Yako Y *et al* (2017) Cell competition with normal epithelial cells promotes apical extrusion of transformed cells through metabolic changes. *Nat Cell Biol* 19: 530 – 541

Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C, Group EGW (2018) Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 29: iv259

Levayer R, Dupont C, Moreno E (2016) Tissue crowding induces caspase-dependent competition for space. *Curr Biol* 26: 670 – 677

Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, Cote JF, Tomasic G, Penna C, Ducreux M *et al* (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 66: 3992 – 3995

Ling S, Hu Z, Yang Z, Yang F, Li Y, Lin P, Chen Ke, Dong L, Cao L, Tao Y *et al* (2015) Extremely high genetic diversity in a single tumor points to prevalence of non-Darwinian cell evolution. *Proc Natl Acad Sci USA* 112: E6496 – E6505

Liu N, Matsumura H, Kato T, Ichinose S, Takada A, Namiki T, Asakawa K, Morinaga H, Mohri Y, De Arcangelis A *et al* (2019a) Stem cell competition orchestrates skin homeostasis and ageing. *Nature* 568: 344 – 350

Liu Z, Yee PP, Wei Y, Liu Z, Kawasawa YI, Li W (2019b) Differential YAP expression in glioma cells induces cell competition and promotes tumorigenesis. *J Cell Sci* 132: jcs225714

Lloyd MC, Cunningham JJ, Bui MM, Gillies RJ, Brown JS, Gatenby RA (2016) Darwinian dynamics of intratumoral heterogeneity: not solely random mutations but also variable environmental selection forces. *Cancer Res* 76: 3136 – 3144

Lu P, Weaver VM, Werb Z (2012) The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol* 196: 395 – 406

Madan E, Parker TM, Pelham CJ, Palma AM, Peixoto ML, Nagane M, Chandaria A, Tomas AR, Canas-Marques R, Henriques V *et al* (2019a) HIF-transcribed p53 chaperones HIF-1alpha. *Nucleic Acids Res* 47: 10212 – 10234

Madan E, Peixoto ML, Dimitrion P, Eubank TD, Yekelchyk M, Talukdar S, Fisher PB, Mi QS, Moreno E, Gogna R (2020) Cell competition boosts clonal evolution and hypoxic selection in cancer. *Trends Cell Biol* 30: 967 – 978

Madan E, Pelham CJ, Nagane M, Parker TM, Canas-Marques R, Fazio K, Shaik K, Yuan Y, Henriques V, Galzerano A *et al* (2019b) Flower isoforms promote competitive growth in cancer. *Nature* 572: 260 – 264

Mamada H, Sato T, Ota M, Sasaki H (2015) Cell competition in mouse NIH3T3 embryonic fibroblasts controlled by Tead activity and Myc. *J Cell Sci* 128: 790 – 803

Mao JH, Wheldon TE (1995) A stochastic model for multistage tumorigenesis in developing and adult mice. *Math Biosci* 129: 95 – 110

Martins VC, Busch K, Juraeva D, Blum C, Ludwig C, Rasche V, Lasitschka F, Mastitsky SE, Brors B, Hielscher T *et al* (2014) Cell competition is a tumour suppressor mechanism in the thymus. *Nature* 509: 465 – 470

Merino MM, Rhiner C, Lopez-Gay JM, Buechel D, Hauert B, Moreno E (2015) Elimination of unfit cells maintains tissue health and prolongs lifespan. *Cell* 160: 461 – 476

Merlo LM, Kosoff RE, Gardiner KL, Maley CC (2011) An in vitro co-culture model of esophageal cells identifies ascorbic acid as a modulator of cell competition. *BMC Cancer* 11: 461

Meyer SN, Amoyel M, Bergantinos C, de la Cova C, Schertel C, Basler K, Johnston LA (2014) An ancient defense system eliminates unfit cells from developing tissues during cell competition. *Science* 346: 1258236

Mitra SK, Schlaepfer DD (2006) Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr Opin Cell Biol* 18: 516 – 523

Morata G, Ripoll P (1975) Minutes: mutants of *Drosophila* autonomously affecting cell division rate. *Dev Biol* 42: 211 – 221

Moreno E, Basler K, Morata G (2002) Cells compete for decapentaplegic survival factor to prevent apoptosis in *Drosophila* wing development. *Nature* 416: 755 – 759

Moreno E, Basler K (2004) dMyc transforms cells into super-competitors. *Cell* 117: 117 – 129

Moreno E, Valon L, Levillayer F, Levayer R (2019) Competition for space induces cell elimination through compaction-driven ERK downregulation. *Curr Biol* 29: 23 – 34.e8

 © 2021 The Authors

Morrissy AS, Garzia L, Shih DJH, Zuyderduyn S, Huang Xi, Skowron P, Remke M, Cavalli FMG, Ramaswamy V, Lindsay PE *et al* (2016) Divergent clonal selection dominates medulloblastoma at recurrence. *Nature* 529: 351 – 357

Moya IM, Castaldo SA, Van den Mooter L, Soheily S, Sansores-Garcia L, Jacobs J, Mannaerts I, Xie J, Verboven E, Hillen H *et al* (2019) Peritumoral activation of the Hippo pathway effectors YAP and TAZ suppresses liver cancer in mice. *Science* 366: 1029 – 1034

Murai K, Skrupskelyte G, Piedrafita G, Hall M, Kostiou V, Ong SH, Nagy T, Cagan A, Goulding D, Klein AM *et al* (2018) Epidermal tissue adapts to restrain progenitors carrying clonal p53 mutations. *Cell Stem Cell* 23: 687 – 699.e8

Nagata R, Nakamura M, Sanaki Y, Igaki T (2019) Cell competition is driven by autophagy. *Dev Cell* 51: 99 – 112.e4

Norman M, Wisniewska KA, Lawrenson K, Garcia-Miranda P, Tada M, Kajita M, Mano H, Ishikawa S, Ikegawa M, Shimada T *et al* (2012) Loss of Scribble causes cell competition in mammalian cells. *J Cell Sci* 125: 59 – 66

Nowell PC (1976) The clonal evolution of tumor cell populations. *Science* 194: 23 – 28

Oertel M, Menthena A, Dabeva MD, Shafritz DA (2006) Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells. *Gastroenterology* 130: 507 – 520. quiz 590

Ohsawa S, Sugimura K, Takino K, Xu T, Miyawaki A, Igaki T (2011) Elimination of oncogenic neighbors by JNK-mediated engulfment in *Drosophila*. *Dev Cell* 20: 315 – 328

Oliver ER, Saunders TL, Tarle SA, Glaser T (2004) Ribosomal protein L24 defect in belly spot and tail (Bst), a mouse minute. *Development* 131: 3907 – 3920

Patel MS, Shah HS, Shrivastava N (2017) c-Myc-dependent cell competition in human cancer cells. *J Cell Biochem* 118: 1782 – 1791

Petrova E, Soldini D, Moreno E (2011) The expression of SPARC in human tumors is consistent with its role during cell competition. *Commun Integr Biol* 4: 171 – 174

Polyak K (2014) Tumor heterogeneity confounds and illuminates: a case for Darwinian tumor evolution. *Nat Med* 20: 344 – 346

Portela M, Casas-Tinto S, Rhiner C, Lopez-Gay JM, Dominguez O, Soldini D, Moreno E (2010) *Drosophila* SPARC is a self-protective signal expressed by loser cells during cell competition. *Dev Cell* 19: 562 – 573

Prober DA, Edgar BA (2000) Ras1 promotes cellular growth in the *Drosophila* wing. *Cell* 100: 435 – 446

Ramon YCS, Sese M, Capdevila C, Aasen T, De Mattos-Arruda L, Diaz-Cano SJ, Hernandez-Losa J, Castellvi J (2020) Clinical implications of intratumor heterogeneity: challenges and opportunities. *J Mol Med* 98: 161 – 177

Rhiner C, Lopez-Gay JM, Soldini D, Casas-Tinto S, Martin FA, Lombardia L, Moreno E (2010) Flower forms an extracellular code that reveals the fitness of a cell to its neighbors in *Drosophila*. *Dev Cell* 18: 985 – 998

Rodrigues AB, Zoranovic T, Ayala-Camargo A, Grewal S, Reyes-Robles T, Krasny M, Wu DC, Johnston LA, Bach EA (2012) Activated STAT regulates growth and induces competitive interactions independently of Myc, Yorkie, Wingless and ribosome biogenesis. *Development* 139: 4051 – 4061

Rosenblatt J, Raff MC, Cramer LP (2001) An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism. *Curr Biol* 11: 1847 – 1857

Sancho M, Di-Gregorio A, George N, Pozzi S, Sanchez JM, Pernaute B, Rodriguez TA (2013) Competitive interactions eliminate unfit embryonic stem cells at the onset of differentiation. *Dev Cell* 26: 19 – 30

Sasaki A, Nagatake T, Egami R, Gu G, Takigawa I, Ikeda W, Nakatani T, Kunisawa J, Fujita Y (2018) Obesity suppresses cell-competition-mediated apical elimination of RasV12-transformed cells from epithelial tissues. *Cell Rep* 23: 974 – 982

Shakiba N, Fahmy A, Jayakumaran G, McGibbon S, David L, Trcka D, Elbaz J, Puri MC, Nagy A, van der Kooy D *et al* (2019) Cell competition during reprogramming gives rise to dominant clones. *Science* 364: eaan0925

Shraiman BI (2005) Mechanical feedback as a possible regulator of tissue growth. *Proc Natl Acad Sci USA* 102: 3318 – 3323

Suijkerbuijk SJ, Kolahgar G, Kucinski I, Piddini E (2016) Cell competition drives the growth of intestinal adenomas in *Drosophila*. *Curr Biol* 26: 428 – 438

Sun Q, Luo T, Ren Y, Florey O, Shirasawa S, Sasazuki T, Robinson DN, Overholtzer M (2014) Competition between human cells by entosis. *Cell Res* 24: 1299 – 1310

Tamori Y, Bialucha CU, Tian AG, Kajita M, Huang YC, Norman M, Harrison N, Poulton J, Ivanovitch K, Disch L *et al* (2010) Involvement of Lgl and Mahjong/VprBP in cell competition. *PLoS Biol* 8: e1000422

van Neerven SM, de Groot NE, Nijman LE, Scicluna BP, van Driel MS, Lecca MC, Warmerdam DO, Kakkar V, Moreno LF, Vieira Braga FA *et al* (2021) Apc-mutant cells act as supercompetitors in intestinal tumour initiation. *Nature* 594: 436 – 441

Vincent JP, Kolahgar G, Gagliardi M, Piddini E (2011) Steep differences in wingless signaling trigger Myc-independent competitive cell interactions. *Dev Cell* 21: 366 – 374

Wagstaff L, Goschorska M, Kozyrska K, Duclos G, Kucinski I, Chessel A, Hampton-O'Neil L, Bradshaw CR, Allen GE, Rawlins EL *et al* (2016) Mechanical cell competition kills cells via induction of lethal p53 levels. *Nat Commun* 7: 11373

Williams MJ, Werner B, Barnes CP, Graham TA, Sottoriva A (2016) Identification of neutral tumor evolution across cancer types. *Nat Genet* 48: 238 – 244

Yamamoto M, Ohsawa S, Kunimasa K, Igaki T (2017) The ligand Sas and its receptor PTP10D drive tumour-suppressive cell competition. *Nature* 542: 246 – 250

Yum MK, Han S, Fink J, Wu SS, Dabrowska C, Trendafilova T, Mustata R, Chatzeli L, Azzarelli R, Pshenichnaya I *et al* (2021) Tracing oncogene-driven remodelling of the intestinal stem cell niche. *Nature* 594: 442 – 447

Zhou Y, Huang T, Cheng AS, Yu J, Kang W, To KF (2016) The TEAD family and its oncogenic role in promoting tumorigenesis. *Int J Mol Sci* 17: 138

Ziosi M, Baena-López LA, Grifoni D, Froldi F, Pession A, Garoia F, Trotta V, Bellosta P, Cavicchi S, Pession A (2010) dMyc functions downstream of yorkie to promote the supercompetitive behavior of hippo pathway mutant cells. *PLoS Genet* 6: e1001140

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